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Nanochannel Trap Arrays for Monitoring Single Mitochondrion Behavior
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We present a nanofluidic device for trapping and fluorescence imaging of individual isolated mitochondria. The device consists of an array of 10 parallel nanochannels fabricated out of PDMS each with a cross section of 500 nm x 2 μm. Mitochondria are isolated from a human cervical cancer cell line (HeLa). Respiration buffer containing the mitochondria is pumped into the device using a syringe pump. Due to the small height of the trap channels individual mitochondria get immobilized one by one along the channels. Time lapsed fluorescence microscopy of JC-1 and TMRM stained mitochondria reveals that trapped mitochondria maintain their membrane potential. The effect of adding substrates and calcium on the membrane potential is studied and the results indicate that mitochondria remain vital and functional in this trapped state. Flickering of membrane potential in some substrate fed mitochondria is observed.

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Nanoplasmonic Optoporation for Large-Scale Precision Gene Regulation in Stem Cells
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One of the key avenues to understanding cell reprogramming is to frequently regulate the genes in individual cells and observe how they interact with each other. Traditional methods for delivery of genetic materials, including viral transduction, lipid-mediated transfection and electroporation, are limited in terms of their temporal and spatial controllability because of bulk processing. Here, we accomplished molecular delivery with single-cell-resolution and the capability of sequential loading in 30 min by nanoplasmonic optoporation. Nanoplasmonic gold nanorods allow us to create transient nanopores (~40 nm diameter) in cell membrane based on the photo-induced nano-scale heating. The lifetime of nanopores is about 30 min. After pore resealing, sequential molecular delivery into embryonic fibroblasts with single-cell precision, as well as at the larger centimeter-scale, is demonstrated. By nanoplasmonic-mediated siRNA deliveries, gene regulation in mouse embryonic stem cells is achieved and shows 26% higher efficiency than conventional lipid-mediated transfection. Large-scale light-controlled gene regulation with single-cell level controllability combined with nanoplasmonic detection (e.g., surface-enhanced Raman scattering and metal-enhanced fluorescence) make NIR-absorbing nanoantennas a promising vector for in situ transfection and real-time monitoring of cell reprogramming.

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Single Cell Electroporation and DNA Dynamics: from Bulk to Micro/Nanolfuidics
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The use of electric pulses to deliver foreign DNA, RNA, proteins or other therapeutic agents into living cells is one of the most popular physical methods for gene delivery. The basic theory of this treatment is that strong electric field can destabilize membranes and induce formation of pores thus increasing membrane permeability. This phenomenon is often known as electroporation (EP), sometimes also referred to electropermeabilization or electric break down. Unfortunately, the fundamental science of the electroporation and transport remains poorly understood, due to the lack of well-defined electroporation conditions on single cells.
In this work, we employ an optical tweezer and micro/nano-fluidics as convenient tools to unravel the physics and mechanism behind the electropermeabilization process in different conditions. We have measured the minimum electric field required for permeabilization of individual single cells in different geometries from bulk to micro and nanoscale for different cell line. In addition, we have measured the minimum electric field required for permeabilization of individual suspended spherical cells as a function of cell diameter for three different cell lines. Key to this work is employing an optical tweezer to manipulate single suspended cells and position in different fluidic geometries (from bulk to nano-scale). Furthermore, we also investigate how DNA move and transport inside these fluidic geometries and deliver into cells. We discovered that delivering a voltage through a nanochannel can generate high electric field over a very small area on the cell membrane, allowing DNAs to be delivered instantly into the cell cytoplasm. In this talk, both experimental work and modeling will be discussed to address the electroporation process.

1139-Plat
At the Nano-Bio Interface: Probing Live Cells with Nano Sensors
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The rapidly evolving field of nanotechnology creates new frontiers for biological sciences. Recently, we and other groups show that vertical nanopillars protruding from a flat surface support cell survival and can be used as subcellular sensors to probe biological processes in live cells. In particular, we are exploring nanopillars as electric sensor, optical sensors, and structural probes. As an electric sensor, nanopillars electrodes offer several advantages such as high sensitivity, subcellular spatial resolution, and precise control of the sensor geometry. A sensitive measurement of cellular electrical activities requires strong coupling between the cell membrane and the recording electrodes. We found that nanopillars electrodes deform the cell membrane inwards and induce negative curvature when the cell engulfs them, leading to a reduction of the membrane-electrode gap distance and a higher sealing resistance. The 3D topology of the nanopillars electrodes is crucial for its enhanced signal detection. A new approach explores nanoelectrodes of a new 3D geometry, namely nanotubes with hollow centers. The nanotube geometry further enhances membrane-electrode coupling efficiency and also significantly increases the time duration of intracellular access. Interestingly, nanopillars serve as focal adhesion points for cell attachment. The presence of high membrane curvature induced by vertical nanopillars or nanotubes affects the distribution of curvature-sensitive proteins. Those studies show a strong interplay between biological cells and nano-sized sensors, which is an essential consideration for future development of interfacing devices.

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Single Cell Response to Periodic Environmental Stimuli using a Microfluidic Bioreactor
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Recent studies have reported the ability of biological systems to implement low-pass filters to distinguish high frequency noise in environmental stimuli from lower frequency input signals. This cellular adaptation is critical for survival in fluctuating environmental conditions, yet we still lack a complete understanding of this phenomenon. Over the last several years, microfluidic-based techniques have been developed to study inducible gene expression at the single cell level, albeit without the ability to control external stimuli with precise methods. Most are limited by long diffusive timescales to alternate environmental concentrations. In this work, we report a microfluidic-based platform for single cell analysis that provides dynamic control over periodic, time-dependent culture media. Single cells are confined in free solution by the sole action of gentle fluid flow, thereby enabling non-perturbative trapping of cells for long time scales. Using fluorescent reporter proteins and cell growth rates as a proxy of cellular fitness, we investigated the effect of small molecule inducers on gene expression of the lac operon in Escherichia coli. Single cell division rates in the microfluidic trap compare favorably to growth rates at room temperature and 37oC measured in batch culture. We observed that single cell gene expression depends on the correlation between growth rate and frequency of exposure to inducer concentrations. In addition, we performed diffusion experiments of TetR:EYFP on a TetO binding array by rapidly switching to concentrations of aTc. Overall, this microfluidic bioreactor provides a direct method for sustaining periodic environmental conditions, measuring growth rates, and detecting gene expression of single cells suspended in free solution.

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Motor-Driven Assembly of Dynamic, Self-Healing Lipid Nanotube Networks
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Interconnected and highly reticulated lipid organelles such as the endoplasmic reticulum play important roles in wide range of physiological processes. While these structures provide a matrix for organizing membrane constituents, the